has been displaced from the substrate molecule indicating more than adequate biological stability of the isotopes in naloxone-7,8-^3H.

Experimental Section§

14-Hydroxymorphinone 3,14-Diacetate (1b). A solution of 1 g of 14-hydroxymorphinone (1a) in 15 ml of acetic anhydride was refluxed for 1 hr. The acetic anhydride was removed under vacuum and the residue was crystallized from ethanol to give 0.93 g of 14-hydroxymorphinone diacetate (1b): mp 248–250°; nmr δ 2.08, 2.27 (acetate methyls), 2.40 (*N*-methyl). *Anal.* ($C_{21}H_{21}NO_6$) C, H, N.

***N*-Cyano-14-hydroxynormorphinone 3,14-Diacetate (1c).** A solution of 0.53 g of the diacetate 1b in 30 ml of $CHCl_3$ was refluxed with 1.5 g of CNBr for 4 hr. After cooling the chloroform was washed with 50 ml of 5% HCl, dried, and evaporated. The residue was crystallized from ethanol to give 0.27 g of *N*-cyano-14-hydroxynormorphinone 3,14-diacetate (1c): mp 243–245°; nmr δ 2.18, 2.25 (acetate methyls), no *N*-methyl absorption. *Anal.* ($C_{21}H_{18}N_2O_6$) C, H, N.

14-Hydroxynormorphinone (1d). A suspension of 100 mg of the *N*-cyano derivative 1c in 10 ml of 20% HCl was heated on a steam bath for 3 hr with continuous stirring. The solution was then evaporated to dryness under a vacuum, and the residue was taken up in 2 ml of H_2O and adjusted to pH 8 with dilute NH_4OH. The aqueous mixture was extracted three times with 5 ml of $CHCl_3$ which was dried and evaporated. The residue showed an equal amount of unsaturated carbonyl absorption at 1680 cm^{-1} and saturated carbonyl at 1730 cm^{-1}, assigned to 14-hydroxynormorphinone and 8,14-dihydroxydihydronormorphinone, respectively. Repeated attempts at separation on various tlc systems failed to effect purification and only increased the intensity of the saturated carbonyl absorption.

Naloxone-7,8-^3H (3b). The above mixture (20 mg) dissolved in 0.5 ml of ethanol containing 20 mg of 10% palladium on charcoal was treated with 5 Ci of tritium gas overnight at room temperature. Labile tritium was removed by dissolving in a small quantity of ethanol and evaporating the solvent, repeating the procedure three times. The residue contained 225 mCi of radioactivity. An aliquot of the above material was taken up in 2 ml of ethanol to which 100 mg of $NaHCO_3$ and 0.20 ml of allyl bromide was added. After refluxing for 18 hr under efficient condensation the reaction mixture was filtered, and the filtrate was taken down to dryness. The residue, containing naloxone-7,8-^3H, was dissolved in 1 ml of ethanol, and an aliquot containing 115,000 cpm was diluted with 53.8 mg of inert naloxone. Recrystallization from ethyl acetate gave specific activities of 917, 827, and 819 cpm/mg, indicating that 39% of the radioactive product was naloxone.

The naloxone-7,8-^3H was purified by preparative tlc on silica gel in the system chloroform–methanol (9:1) containing 4 drops of NH_4OH. The area corresponding to standard naloxone which was run alongside was eluted with ethanol–methylene chloride. One portion of the eluted material was chromatographed on thin-layer silica gel in the system ethanol–acetic acid–water (60:30:10). After development the plate was scanned for radioactivity, showing only one radioactive peak at R_f 0.5 corresponding to standard naloxone which was run alongside.

Another portion of the purified naloxone-7,8-^3H containing 40,800 cpm was diluted with 29.6 mg of inert naloxone. Recrystallization from ethyl acetate gave the following successive specific activities: 1349, 1305, 1383 cpm/mg. The average specific activity of 1346 cpm/mg corresponds to a total of 39,840 cpm or 97.5% of the radioactivity present.

The naloxone-7,8-^3H could also be purified on a Celite partition column with 90% methanol–10% water as the stationary phase and 100% isooctane followed by a 25% dichloroethane gradient as the eluting solvents. The 10-ml fractions were obtained by an automatic fraction collector and aliquots were removed for counting. Naloxone was eluted with 100% isooctane as a single radioactive peak in fractions 25–44. After introduction of a 25% dichloroethane gradient at fraction 100, another radioactive peak in fractions 205–225 was obtained and was presumed to consist of 14-hydroxydihydronormorphinone-7,8-^3H which had failed to react with the allyl bromide. The naloxone-7,8-^3H purified by this procedure was also 98% pure by reverse isotope dilution.

§ Nmr spectra were obtained in deuteriochloroform on a Varian A-60 instrument. Infrared spectra were obtained in KBr solutions on a Beckman IR 13 spectrometer. Analyses are by Spang Laboratories. Counting was carried out either in toluene or in Diotol using a Packard scintillation counter.

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New 5-Substituted 1-Alkyl-2-nitroimidazoles

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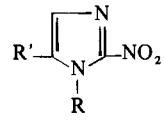
During the course of a literature survey we have noticed that although there is much information on the well-known antiprotozoal activity of 2-nitroimidazoles, very little data on the antibacterial and antifungal activity are available.

According to Nakamura,[1] 2-nitroimidazole (azomycin) itself mainly inhibits the *in vitro* growth of gram-negative bacteria (*Escherichia coli*, MIC 25 μg/ml), whereas it is completely inactive against fungi. Beaman[2] reported that, at a concentration of 5 μg/ml, azomycin inhibits *Pseudomonas aeruginosa*, *E. coli*, and *Staphylococcus aureus*, 1-methylazomycin inhibits yeasts and 1-allylazomycin *E. coli* only, whereas 4,5-dimethylazomycin is completely inactive. Grunberg,[3] examining the protective effect against lethal systemic infections in mice of a series of 2-nitroimidazoles carrying in position 1 alkyl substituents with amido or hydroxy functions, revealed no activity against *P. aeruginosa* and *Proteus vulgaris*, a weak activity against *E. coli* and *Streptococcus pyogenes*, and a consistent activity against *S. aureus*. Prince[4] found that 1-(3-methoxy-2-hydroxypropyl)-2-nitroimidazole possesses a weak *in vitro* activity against *E. coli* (MIC 250 μg/ml) but it is practically ineffective against *S. aureus*, *S. pyogenes*, *P. aeruginosa*, and *P. vulgaris*.

We have tested for their *in vitro* activity on a series of gram-negative and gram-positive bacteria and fungi about 30 variously substituted 2-nitroimidazole derivatives previously examined for their antitrichomonas activity.[5] Compounds possessing antimicrobial activity below concentrations of 200 μg/ml are shown in Table I. The only correlation between antibacterial and antitrichomonas activity is for activity against *Clostridium perfringens*, as already noted by Prince[4] with similar nitroimidazoles. The most active compounds **14–16** showed no protection in experimental infection in mice against *E. coli* at 60 mg/kg.

Therefore, in order to extend the study on 2-nitroimidazole derivatives, it seemed interesting to introduce in posi-

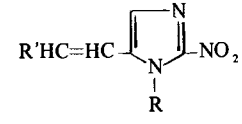
Table I. *In Vitro* Activity of Some 2-Nitroimidazoles against Selected Organisms



Compd	R	R'	Minimum inhibitory concentration, μg/ml[a,b] St.a.	St.h.	Cl.p.	E.c.	Ps.a.	T.m.	My.t.	T.v.[c] Static	"Cidal"
1	CH_2CH_2-c-$N(CH_2CH_2)_2O$	H	>20[d]		20	>20[e]	>20	>20[f]	>20	>100	
2	H	*n*-C_3H_7	>20[d]			10[e]	>20	>20[f]	>20	10	20
3	CH_3	CH_3	50[d]	>200		>200	>200	>200	>200	5	10
4	CH_3	C_2H_5	>200	>200	5	>200	>200	>200	>200	10	20
5	CH_3	*i*-C_3H_7	>20[d]		5	>20[e]	>20	>20[f]	>20	5	20
6	CH_3	CH_2CH_2OH	200		1	50	>200	>100	>200	20	50
7	CH_3	CH_2CH_2Cl	>200			200	200	200	200	10	50
8	C_2H_5	CH_2CH_2Cl	>100	>100	5	>100	>100	>100	20	10	20
9	CH_3	$CH_2CH(OH)CH_3$	>200			50	>200	>200	>200	10	20
10	CH_2CH_2OH	C_2H_5	200		2	50	>200	>200	>200	20	50
11	CH_2CH_2OH	*n*-C_3H_7	>200			100	>200	>200	200	2	10
12	CH_2CH_2OH	*i*-C_3H_7	>200			50	>200	>200	>200	10	50
13	CH_2CH_2Cl	CH_3	>200			200	>200	>200	200	5	10
14	CH_2CONH_2	CH_3	50	20	10	20[e]	>200	>200	>100	>100	
15	$CH_2CONHCH_3$	CH_3	100			20	>200	>200	>200	>100	
16	$CH_2CONHCH_3$	C_2H_5	>100	50	0.5	5[e]	>200	>200	>200	50	100

[a]See footnote †. [b]St.a. = *Staphylococcus aureus* T, St.h. = *Streptococcus hemolyticus* C203, Cl.p. = *Clostridium perfrigens* ISS 30453, E.c. = *Escherichia coli* SKF 12140, Ps.a. = *Pseudomonas aeruginosa* ATCC 10145, T.m. = *Trichophyton mentagrophytes* SKF 17410, My.t. = *Mycobacterium tuberculosis* H37Rv ATCC 9360, T.v. = *Trichomonas vaginalis*. [c]Metronidazole: § minimal trichomonastatical concentration 0.5–1 μg/ml, minimal trichomonacidal concentration 2–10 μg/ml. [d]*Staphylococcus aureus* ATCC 6538. [e]*Escherichia coli* ATCC 10536. [f]*Trichophyton mentagrophytes* ATCC 8757.

Table II. 1-Alkyl-2-nitro-5-vinylimidazoles



Compd	R	R'	*In vitro* activity against selected organisms, MIC, μg/ml[a,b] St.a.	St.h.	D.pn.	Cl.p.	P.v.	E.c.	Sh.s.	S.ty.	K.pn.	Ps.a.	C.a.	T.m.	My.t.	Myc.g.	T.v. Static	"Cidal"
17	CH_3	H	50	>100	>100	0.5	100	10	20	5	10	20	100	50	20	50[c]	1	1
18	C_2H_5	H	100	>100	>100	0.5	>100	20	50	10	20	>100	>100	50	5	>100	1	5
19	CH_3	C_6H_5	>20[d]	>20	>20	1	>20	>20	>20	>20	>20	>20	>20	>20	>20		2	10
20	C_2H_5	C_6H_5	>20[d]	>20	20	1	>20	>20	>20	>20	>20	>20	>20	>20	10	>20[c]	5	20

[a]See footnotes *a–c*, Table I. [b]D.pn. = *Diplococcus pneumoniae* UC41, P.v. = *Proteus vulgaris* X19 ATCC 881, Sh.s. = *Shigella sonnei* ATCC 9290, S.ty. = *Salmonella typhimurium* Kh, K.pn. = *Klebsiella pneumoniae* ISM, C.a. = *Candida albicans* SKF 2270, Myc.g. = *Mycoplasma gallisepticum* H21 CZB. [c]*Mycoplasma gallisepticum* S6 CAPM 5128. [d]Higher concentrations not proved due to the poor solubility of the compounds in DMF.

tion 5 unsaturated conjugated groups (vinyl, styryl) or functions at different levels of oxidation.

Chemistry. The synthesis and the chemicophysical characteristics of the compounds not described in detail in the Experimental Section has been previously reported.[6]

The 1-alkyl-2-nitro-5-vinylimidazoles (**17** and **18**) were obtained from the corresponding 1-alkyl-2-nitro-5-(2-chloroethyl)imidazoles[5,6] by treatment with potassium *tert*-butoxide. The oxidation of compound **17** with neutral potassium permanganate gave the diol **24**. The 1-alkyl-2-nitro-5-(2-phenylvinyl)imidazoles (**19** and **20**) were prepared by condensation of the corresponding 1-alkyl-2-nitro-5-methylimidazoles[5] with benzaldehyde in the presence of potassium *tert*-butoxide. The trans configuration was supported by nmr spectra examination. The 1-methyl-2-nitro-5-hydroxymethylimidazole (**21**) was prepared from the nitroimidazolecarboxaldehyde **22** by reduction with $NaBH_4$. The 1-methyl-2-nitro-5-acetylimidazole (**25**) was obtained by treating compound **22** with CH_2N_2. The hydrolysis of 1-methyl-2-nitro-5-carbethoxyimidazole (**28**) gave the corresponding 1-methyl-2-nitroimidazole-5-carboxylic acid (**26**) which was transformed by CH_2N_2 into the methyl ester **27**.

Screening Results.† The 5-vinyl derivative **17** (Table II) showed a wide *in vitro* activity against gram-negative bacteria and a certain degree of activity against fungi. This biological activity was considerably lower in compound **18**, possessing an ethyl chain in position 1, except for *Mycobacterium tuberculosis*. The styryl derivatives **19** and **20** were inactive at 20 μg/ml on the selected organisms. Due to the poor solubility, in DMF higher concentrations could not be tested.

The expected *in vitro* "cidal" activity on *Trichomonas vaginalis* was also slightly decreased for the 1-ethyl derivatives **18** and **20**. In the experimental infection with this organism in mice, compound **17** possessed an ED_{50} po of 34.9 mg/kg (metronidazole,‡ ED_{50} po 11.5 mg/kg) whereas it was inactive at 200 mg/kg in an *E. coli* infection. Compounds **19** and **20** were found inactive against *T. vaginalis* at 40 mg/kg in the experimental infection in mice. The 5-styryl derivative **19** was more toxic (LD_{50} po 168.0 mg/kg) than the corresponding 5-vinyl derivative **17** (LD_{50} po 480.0 mg/kg).

The 1-methyl-2-nitroimidazole-5-carboxaldehyde (**22**) was found to possess a broad spectrum *in vitro* activity (Table III). A significant decrease in activity, particularly against fungi, was caused by the substitution of the 5-aldehydo by a keto (**25**) or by a 5-carbalkoxy function (**27** and **28**). The 5-hydroxymethyl (**21**) and 5-carboxy (**26**) substituted compounds showed no detectable activity.

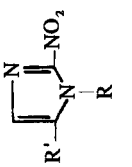
The *in vitro* activity against *E. coli* of **22** was not maintained in experimental infection in mice since no protection was found at 100 mg/kg po (LD_{50} po 412.0 mg/kg). It was observed that **22** was not inactivated by bovine serum, while a certain degree of inactivation was showed by **27** (MIC 100 μg/ml on *S. aureus* T + serum); the vinyl derivative **17** was unaffected by serum.

All the compounds listed in Table III were less active than most other 2-nitroimidazoles against *T. vaginalis in vitro*. Once more, a good correlation between antitrichomonas and anticlostridium activity was shown for the nitroimidazole derivatives included in Tables II and III.

†The *in vitro* and *in vivo* biological activity data were obtained using methods previously described (*cf.* ref 5, 7, and 8). The LD_{50} values were calculated according to the method of Litchfield and Willcoxon.[9]

‡1-(2-Hydroxyethyl)-2-methyl-5-nitroimidazole.

Table III. 5-Functionally Substituted 1-Alkyl-2-nitroimidazoles



			In vitro activity against selected organisms, MIC, μg/ml[a]											T.v.	
Compd	R	R′	St.a.	St.h.	D.pn.	Cl.p.	P.v.	E.c.	Sh.s.	S.ty.	K.pn.	Ps.a.	C.a.	T.a.	My.t.	Myc.g.	Static	"Cidal"
21	CH_3	CH_2OH	100	>100	100	1	>100	>100	>100	100	>100	>100	>100	>100	>100	100[b]	20	50
22	CH_3	CHO	50	50	20	10	50	10	20	2	10	10	50	10	10	100[b]	100	>100
23	C_2H_5	CHO	50	100	20	10	100	5	20	50	100	>100	>100	50	10	50	20	50
24	CH_3	$CH(OH)CH_2OH$	>20[c]	>20	>20	20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20[b]	100	>100
25	CH_3	$COCH_3$	50	100	20	20	100	10	50	100	100	>100	>100	50	50	100	50	>100
26	CH_3	COOH	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	
27	CH_3	$COOCH_3$	50	>100	50	5	>100	20	100	100	>100	>100	>100	>100	>100	>100	>50	
28	CH_3	$COOC_2H_5$	100	100	50	20	>100	20	100	>100	>100	>100	>100	>100	50	>100[b]	>100	

[a]See footnotes *a* and *b*, Table II. [b]*Mycoplasma gallisepticum* S6 CAPM 5128. [c]Higher concentrations not proved due to the poor solubility in DMF.

Experimental Section§

trans-1-Ethyl-2-nitro-5-(2-phenylvinyl)imidazole (20). This compound was prepared from 1-ethyl-2-nitro-5-methylimidazole[5] by the procedure previously described[6] for the 1-methyl analog. Recrystallization from *i*-PrOH afforded a product melting at 154–156° (8%); tlc R_f 1.12 (relative to the starting compound); ir 1530 (ν_{asym} NO_2), 1380 (ν_{sym} NO_2), 960 (γ CH trans), 835 (skeletal imidazole), 758 and 695 cm^{-1} (γ CH phenyl); nmr δ 1.50 (t, 3 H, CH_3), 4.55 (q, 2 H, CH_2), 6.82 (d, 1 H, $J_{CH=CH}$ = 16 Hz, =CHCN), 7.23 [d, 1 H, =CH(C_6H_5)], 7.20–7.75 (m, 6 H, ring H and arom H). *Anal.* ($C_{13}H_{13}N_3O_2$) C, H, N.

1-Methyl-2-nitro-5-hydroxymethylimidazole (21). A solution of 1.9 g (0.05 mol) of $NaBH_4$ in 150 ml of EtOH was added to a solution of 1.55 g (0.01 mol) of 1-methyl-2-nitroimidazole-5-carboxaldehyde (22)[6] in 200 ml of EtOH with stirring, while the temperature was maintained at −5°. The reaction was monitored by tlc. When the reaction was completed the excess of $NaBH_4$ was decomposed by adding 10% HCl at 0°. After filtering, the solvent was removed and the residue was extracted with Me_2CO. The extracts were concentrated to a small volume. After standing at 4°, 1 g of product (63.6%), mp 142–144°, was obtained: tlc R_f 0.60 (relative to 22); the product was identical (ir and nmr spectra) with a sample obtained[6] by $LiBH_4$ reduction of 1-methyl-2-nitro-5-carbethoxyimidazole (28).

1-Methyl-2-nitro-5-acetylimidazole (25). An anhydrous ethereal solution (86 ml) of CH_2N_2 (5 mmol) was added with cooling to a solution of 0.7 g (4.5 mmol) of 22 in 180 ml of anhydrous Et_2O. After standing for 24 hr at room temperature an additional amount of CH_2N_2 (5 mmol) was added, and the mixture was left to stand for 48 hr. The reaction mixture was evaporated to dryness and the residue (0.62 g) was dissolved in $CHCl_3$ (6 ml) and applied to six preparative chromatographic plates (20 × 20 cm).

After developing, the silica gel corresponding to the zone with R_f 0.64–0.75 was collected and eluted with MeOH. By evaporation to a small volume, a crystalline compound was obtained: 65 mg (8.5%); mp 81–83°; tlc R_f 1.20 (relative to 22); ir 1670 (ν C=O), 1520 (ν_{asym} NO_2), 1350 (ν_{sym} NO_2), 935 (γ CH), 837 cm^{-1} (skeletal imidazole); nmr δ 2.68 (s, 3 H, CH_3CO), 4.33 (s, 3 H, CH_3N), 7.85 (s, 1 H, ring H). *Anal.* ($C_5H_7N_3O_3$) H, N; C: calcd, 42.61; found, 41.92.

1-Methyl-2-nitroimidazole-5-carboxylic Acid (26). A mixture of 1.4 g (7 mmol) of 1-methyl-2-nitro-5-carbethoxyimidazole (28)[6] and 8 g of NaOH in 90 ml of H_2O was heated for 20 min until an homogeneous solution was obtained.

After cooling, the reaction mixture was acidified to Congo red with 10% HCl and evaporated to dryness. The residue was extracted with EtOAc. The solution upon concentration gave 0.6 g (50%) of crystals: mp 161–163°; tlc R_f 0.10 (relative to 28); ir 2700–2100 (ν OH), 1720 (ν C=O), 1530 (ν_{asym} NO_2), 1360 (ν_{sym} NO_2), 1240 (ν CO), 970 (γ OH), 840 cm^{-1} (skeletal imidazole); nmr (DMSO-d_6) 4.20 (s, 3 H, CH_3N), 7.75 (s, 1 H, ring H), 10.5–13.5 (broad, 1 H, COOH); uv λ max, nm (log ε) 305 (3.80), 243 (3.77). *Anal.* ($C_5H_5N_3O_4$) C, H, N.

1-Methyl-2-nitro-5-carbomethoxyimidazole (27). This compound was prepared by treating a solution of 1.1 g of 26 in 500 ml of Et_2O with an ethereal solution of CH_2N_2. After recrystallization from *i*-PrOH–(*i*-Pr)$_2$O, 0.7 g (58%) of 27, mp 57–58°, was obtained: tlc R_f 1.3 (relative to 26); ir 1730 (ν C=O), 1520 (ν_{asym} NO_2), 1360 (ν_{sym} NO_2), 1240 and 1105 (ν CO), 965 (γ CH), 840 cm^{-1} (skeletal imidazole); nmr δ 3.96 (s, 3 H, $COOCH_3$), 4.25 (s, 3 H, CH_3N), 7.73 (s, 1 H, ring H). *Anal.* ($C_6H_7N_3O_4$) C, H, N.

The synthesis by a different route of compound 27 has been reported by Asato and Berkelhammer[10] after this manuscript had been sent for publication.

Acknowledgment. We are indebted to Professor L. Silvestri for helpful discussions.

§Melting points (uncorrected) were determined in open capillary tubes. Ir spectra were determined with a Perkin-Elmer Model 137 spectrophotometer as Nujol mulls. Nmr spectra were recorded at 60 MHz by a Varian A-60 spectrometer in $CDCl_3$ except when otherwise indicated. Chemical shifts are reported as δ relative to TMS (δ 0.00 ppm). Uv spectra were recorded with a Unicam S.P. 800 spectrophotometer. Thin-layer chromatograms were run on silica gel HF-uv_{254} plates to a distance of 10.0 cm (developed with a 1:9 mixture of MeOH and $CHCl_3$). The spots were detected by visual examination under uv light. Evaporation of solvents was done under reduced pressure using a rotary evaporator. Where analyses are indicated only by symbols of the elements, analytical results for those elements were within ±0.4% of the theoretical values.

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Agents Acting on the Central Nervous System. 15. 2-Substituted 1,2,3,4,6,7,12,12a-Octahydropyrazino[2′,1′:6,1]pyrido[3,4-*b*]indoles. A New Class of Central Nervous System Depressants†

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In continuation to our earlier work on piperazines in a rigid framework,[1] 1,2,3,4,6,7,12,12a-octahydropyrazino-[2′,1′:6,1]pyrido[3,4-*b*]indole (I, R = H), a ring system which incorporates both tryptamine and piperazine and also is structurally related to oxypertine,[2,3] a major tranquilizer, has been synthesized along with a number of 2-substituted derivatives and evaluated for their pharmacological activities. The results are reported in this communication.

During the course of this work Schulenberg and Page[4] reported the 2-phenyl derivative of I and found it to be devoid of any useful biological activity; the parent nucleus (I, R = H) was not synthesized. The new synthesis now reported for I (R = H) is more convenient and gave better yields, and the compounds reported show marked tranquilizing activity.

Two methods were used to synthesize I. In the first method, which was generally used in this work, I was synthesized starting from *dl*-tryptophane which on cyclization with formaldehyde followed by esterification gave **2**, which on condensation with ethyleneimine gave the lactam **4** in 66% yield. The lactam **4** on $LiAlH_4$ reduction in THF gave **6** (80%), the ir of which was characterized by Bohlman bands[5,6] at 2700–2800 cm^{-1}, indicating a trans ring junction. This synthesis of **6** is stereospecific since starting from *l*-tryptophane, optically active lactam **3** and tetracyclic base **5** could be obtained; the chiral center 12a- in (−)-**3** and (−)-**5** would have an *S* configuration as present in (−)-tryptophane. A large variety of substituents were introduced at the 2 position of **6** by methods described in the Experimental Section to give I.

The second approach to the synthesis was essentially on the lines described by Schulenberg and Page.[4] Thus, con-

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